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The evaluation of inflammation induced by material implanted subcutaneously in the rat

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Inflammation induced by cotton pellets, implanted subcutaneously in the rat, has been compared with that resulting from implantation of pellets impregnated with irritant substances. Time sequences of fluid exudation and granulation tissue formation have been evaluated by differential weighing. Increased irritancy of the pellets resulted in increased exudate volume, and heavier granulation deposition. Contact between granulation tissue and irritant pellets was delayed until the volume of exudate had subsided. Penetration of the pellet was also delayed. Systemic anti-inflammatory effects from granulomata surrounding implanted pellets were related to either the size of the implantation lesion or the degree of irritancy of the implanted material. Evaluation of granulation tissue formation is suggested as a test for irritancy of surgical fabrics.

The nature of the inflammatory response to subcutaneously implanted foreign material has been investigated mainly in experiments applied to the evaluation of anti-inflammatory drugs (Meier, Schuler & Desaulles, 1950; Eichhorn & Sniffen, 1964), the properties of materials used in prosthetic surgery (Newman, 1956; Arons, Sabesin & Smith, 1961), or the toxicity of plastics materials (U.S. Pharmacopeia, 1965; Lawrence, Mitchell & others, 1963). Such investigations may also be of value in assessing the relative degree of irritancy of other materials which might come into contact with tissue, e.g. surgical fabrics. For this purpose both qualitative and quantitative aspects of the tissue reactions to implanted irritant material have been studied. In addition, the systemic anti-inflammatory influence of one irritant lesion upon others has been investigated as a possible parameter for the assessment of irritant potency.

EXPERIMENTAL AND RESULTS

Material and Methods

Male Wistar rats weighing 200–250 g, were used. They were housed in well ventilated conditions with the temperature regulated at 65° F, and were maintained on unrestricted supplies of water and diet 41B (Oxoid).

Material for implantation. Either weighed portions of absorbent gauze B.P.C. (40 mg prepared as pellets by rolling vigorously between gloved fingers) or selected cotton wool dental pellets (Johnson & Johnson) (8 mg) were used. Pellets, with incorporated irritant substances, were similarly prepared from euflavine gauze B.P.C. 1954, boric acid gauze B.P.C. 1954, or iodoform gauze B.P.C. 1954, and also from dental pellets or absorbent gauze B.P.C. repeatedly soaked and dried in stronger tincture of capsicum B.P.C. 1934. Pellets were initially sterile and aseptic precautions were taken in their handling.

Implantation. One or more pellets were introduced in each animal under light

ether anaesthesia. The pellets were placed subcutaneously in a lateral abdominal position, either through a dorsal midline incision, or by means of a pellet implantation instrument inserted in the groin. Midline incisions were sealed with suture clips.

After periods varying from 3 to 56 days animals carrying implants were killed by a blow on the head and the pellets, together with surrounding granulation tissue, were dissected and removed. Each pellet and associated tissue was weighed wet, and then dried to a constant weight at 60° and reweighed. Where considerable accumulation of exudate fluid had occurred, the fluid was collected with a pasteur pipette and subsequently replaced with the pellet for weighing.

When dissecting, granulation tissue was identified as that firm vascular tissue surrounding a cavity filled with exudate, or adherent to the inserted pellet. It was generally well differentiated from normal areolar connective tissue. In sample cases the accuracy of dissection was confirmed histologically.

Time course of development of exudate and granulation tissue

Groups of five rats were selected at random and two identical cotton dental pellets were implanted one on each side in every animal. After given periods of time all animals in each group were killed and the pellets were removed. Pellets from four of the animals were weighed and those from the remaining rat in each group were examined histologically. Other groups of five rats were similarly implanted with cotton dental pellets impregnated with capsicum oleoresin, and the pellets were examined after corresponding periods of time. Wet and dry weights were as shown in Table 1.

The sequences of qualitative changes which occurred after the implantation of these pellets are illustrated diagrammatically in Fig. 1. These changes can conveniently be considered as divided into three phases: namely exudation, granulation and consolidation.

Exudative phase. At three days after implantation there was a marked rise in the wet weight of the normal pellets, attributable to the accumulation of a visible fluid exudate. The exudate filled a sac around each pellet, but at this time it was impossible, on the basis of naked-eye observation, to decide the boundary of the sac. The fluid was protein rich and contained numerous polymorphonuclear leucocytes—largely neutrophils. Subsequently the wet weights fell as the exudate was resorbed. After

Table 1. *Weights of granulation tissue at various times after subcutaneous implantation of cotton pellets, compared with tissue from pellets impregnated with capsicum oleoresin.* Original pellet weights 8 mg. Each value represents the mean from eight pellets

Days after implantation	Mean granulation tissue weight (mg) ± standard errors			
	Normal pellets		Impregnated pellets	
	Wet	Dry	Wet	Dry
3	79·8 ± 28·5	4·6 ± 1·9	130·2 ± 36·0	10·3 ± 3·1
7	108·0 ± 16·3	15·3 ± 1·6	166·4 ± 40·5	20·3 ± 2·6
13	97·9 ± 12·4	20·2 ± 1·9	173·8 ± 29·6	25·8 ± 4·8
20	96·0 ± 8·9	19·2 ± 2·3	150·0 ± 18·2	29·4 ± 1·8
28	59·5 ± 6·7	24·9 ± 2·0	105·2 ± 20·4	32·6 ± 2·6
42	46·1 ± 6·8	17·4 ± 1·5	78·0 ± 10·4	44·6 ± 4·8
56	42·5 ± 5·1	17·2 ± 1·2	64·9 ± 14·2	48·2 ± 3·3

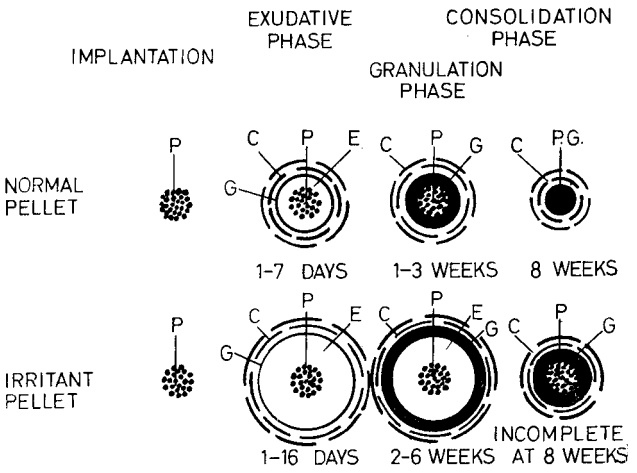

FIG. 1. Diagrammatic comparison of development of inflammation and granulation tissue, induced by implantation of non-irritant or irritant pellets. Key: P, pellet; C, normal connective tissue; G, granulation tissue; E, exudate.

seven days the granulation layer was discernible although this having scattered capillaries and a sparse layer of fibroblasts and collagen fibres.

The oleoresin-impregnated pellets provoked a much more severe and prolonged exudative reaction. The volume of exudate was higher than that from the normal pellets after three days, and increased further for up to two weeks. At seven days the granulation layer was thin and not well differentiated from surrounding tissue. No tissue adhered to the pellet for up to 28 days.

Granulation phase. At seven days the granulation tissue was loosely adherent to the normal cotton pellets. The granulation layer thickened during the following week, and its capillary network became more complex with noticeable budding of new vessels towards the pellet. Fibroblast and macrophage numbers had markedly increased with a thickening of the web of collagen fibres. Some penetration of outermost layers of the pellets had occurred and the tissue was firmly adherent. By 21 days after implantation, macrophages and fibroblasts had reached deeper layers, collagen fibres were deeply enmeshed with the cotton fibres, and capillaries were established in the fibre network at the pellet surface. During this period the wet weights had markedly declined and the dry weights approached maximal values. No significant increase in dry weights occurred after 21 days.

In contrast the granulation layer around the impregnated pellets was less well defined between 7 and 14 days and the pellets still lay unattached in sacs of exudate. Subsequently the granulation layer became much more apparent and developed in considerable thickness around the pellet, replacing the exudate, and then adhering to the pellet. A steady decline in wet weights, commencing after two weeks and accompanied by a sustained rise in dry weights, marked the development of granulation tissue much thicker than around normal cotton pellets. More fibroblasts and leucocytes were present and the capillary and collagen development was much denser.

Consolidation phase. For normal pellets observed after week three, there was a small decrease in dry weight of material deposited. This was accompanied by further decrease in wet weights. Histological changes occurring during this time

were further penetration of fibroblasts and collagen fibres to the centres of the pellets, and some giant cell formation around the cotton fibres. Thickness and vascularity of the outer layers decreased leaving a thinner but denser fibre barrier. After eight weeks numbers of fibroblasts and leucocytes had decreased in all fields.

Up to the end of the period investigated, penetration of impregnated pellets was poor, so that most of the granulation tissue remained in a weakly adherent but thick layer at the surface. Large numbers of leucocytes and fibroblasts clustered on and just within the outer interstices of the pellets.

Comparison of the effects of various impregnating substances on fabric implant induced granulation tissue

Using light ether anaesthesia, 40 mg pellets of absorbent gauze B.P.C., or of other gauze fabrics impregnated with euflavine, boric acid, iodoform, or capsicum oleoresin, were inserted. Groups of five rats were used, each animal receiving two pellets of the same type of fabric. Eleven days later each animal was killed and pellets with surrounding granulation tissue were dissected and removed. Wet and dry weights of granulation tissue were found, the amount in excess of 40 mg representing the weight of exudate or granulation tissue. Results were as shown in Table 2. In a separate experiment similar clean or impregnated fabric pellets were implanted for 11 days, but the pellets were dissected out with only tissue directly adherent to the pellets. The dry weights of such material were measured, and were as shown in Table 2.

Table 2. *Dry (I) and wet (II) weights of granulation tissue surrounding pellets and (III) adherent to pellets of gauze fabrics implanted subcutaneously for* 11 *days.* Dry weight of pellet (40 mg) subtracted from each weighing

	I			II			III		
Fabric	Mean wt (g)	Standard error	No. of observations	Mean wt (g)	Standard error	No. of observations	Mean wt (g)	Standard error	No. of observations
Absorbent gauze.. .. (B.P.C.)	0·1238	±0·015	10	0·636	±0·057	10	0·099	±0·010	10
Euflavine gauze .. .. (B.P.C. 1954)	0·1523	±0·010	8*	0·804	±0·060	8*	0·042	±0·012	5*
Boric acid gauze .. .. (B.P.C. 1954)	0·1487	±0·006	10*	0·771	±0·043	10*	0·064	±0·05	10*
Iodoform gauze .. .. (B.P.C. 1954)	0·1644	±0·012	8*	0·890	±0·201	—	0·071	±0·008	10*
Gauze impregnated with capsicum oleo resin ..	0·252	±0·028	8†	1·368	±0·089	8*†	No adherent tissue		5*

* Denotes significant difference from absorbent gauze ($P < 0·05$).
† Denotes significant difference from all other fabrics ($P < 0·05$).

It can be seen that after implantation for 11 days both the dry and wet weights of material deposited around the impregnated pellets, were significantly higher ($P < 0·05$) than those of tissue on pellets of absorbent gauze B.P.C. Weights of granulation tissue actually adhering to the pellets were significantly lower ($P < 0·05$) in the case of impregnated pellets.

Wet and dry weights of granulation tissue surrounding pellets impregnated with capsicum oleoresin were significantly higher ($P < 0·05$) than those associated with any of the other fabrics. There was no tissue directly adherent to these pellets.

Table 3. *Granulation tissue deposited on single "target" cotton pellets in animals with simultaneous implantation of four other pellets.* Dry weight of target pellet (8 mg) subtracted from each weighing. Each value represents the mean weight (mg ± s.e.) from five pellets

	Time of implantation			
	2 days	5 days	14 days	49 days
Wet weights (mg)				
Control—target pellet alone ..	239 ± 46	188 ± 19	128 ± 10	95 ± 13
Test—plus 4 other pellets ..	138 ± 21*	144 ± 15*	102 ± 6*	91 ± 10
Dry weights (mg)				
Control—target pellet alone ..	5·7 ± 1·2	10·7 ± 1·4	22·6 ± 2·0	19·9 ± 3·3
Test—plus 4 other pellets ..	3·4 ± 1·5	6·2 ± 2·0	15·3 ± 1·2	18·9 ± 2·4

* Denotes significant difference between means.

Anti-inflammatory influence of simultaneously implanted irritant material

Single cotton pellets (8 mg) were implanted in the lateral abdominal subcutaneous connective tissue on the left side of each animal in groups of five rats (*target pellets*). At the same time four pellets were implanted together on the opposite side of each of the animals (*test pellets*). Groups of animals thus treated were killed after 2, 5, 14 or 49 days, and the single target pellets, together with surrounding granulation tissue, were dissected and removed. Other groups of animals received only the single target pellets in the left flank; the implanting instrument being inserted on the opposite side but no pellets deposited. Wet and dry weights of the target granulation tissue were determined and the growth in presence or absence of other granuloma inducing material was as shown in Table 3. It can be seen that the development of both exudate and granulation tissue was retarded in those animals implanted with additional granuloma inducing material. This effect was observable over the first 14 days of the inflammatory reaction. To determine whether the degree of inhibition exerted by one granulation reaction upon another was dependent upon the relative size of the two sites of inflammation, the previous experiment was repeated with varying numbers of "test" pellets. Single pellets were implanted in the left flank of rats in groups of 5, while 1, 2, 4 or 8 pellets were simultaneously implanted on the opposite sides. One group received only single pellet implantations and these acted as control animals. Five days later the rats were killed and the target pellets removed and weighed. Results were as shown in Table 4. It was found that the degree of inhibition increased

Table 4. *Granulation tissue deposited on single "target" cotton pellets in rats with simultaneous implantation of additional pellets.* Dry weight of target pellet (8 mg) subtracted from each weighing. Implantation for five days.

	Mean "target" granulation tissue weight (mg) ± s.e.	
Number of "test" pellets	Wet	Dry
0	201 ± 37	11·2 ± 2·3
1	194 ± 18	11·8 ± 2·0
2	174 ± 26	7·8 ± 2·0
4	146 ± 20	4·0 ± 1·0
8	140 ± 16	6·4 ± 1·0

Table 5. *Granulation tissue deposited on single cotton "target" pellets, influenced by presence of "test" pellets* (40 *mg*) *impregnated with irritant substances* Implantation for 5 days. Dry weight of "target" pellet (8 mg) subtracted from each weighing.

Type of "test" pellets	Mean "target" granulation tissue weight (mg) ± s.e.	
	Wet	Dry
None (control)	145 ± 22	23·9 ± 2·1
Absorbent gauze	90 ± 10*	16·2 ± 2·4*
Capsicum gauze	63 ± 12*†	11·7 ± 2·2*†
Euflavine gauze	76 ± 10*	10·6 ± 1·9*†

* Denotes value significantly different ($P < 0{\cdot}05$) from corresponding control value.
† Denotes value significantly different ($P < 0{\cdot}05$) from corresponding result for absorbent gauze B.P.C.

with the number of "test" pellets. It was noted that although larger areas were involved the inflammatory reactions surrounding multiple pellets were qualitatively similar.

Evaluation of the indirect antigranulomatous influence of implants impregnated with irritant material. Single cotton dental pellets (8 mg) were implanted in the left side abdominal subcutaneous connective tissue of rats. Groups of five of these animals were implanted, at the same time, with single 40 mg pellets of either absorbent gauze B.P.C., euflavine gauze, or gauze impregnated with capsicum oleoresin, subcutaneously on the right side. Eight days after implantation the animals were killed and the target pellets and granulation tissue removed and weighed. Wet and dry weights were as shown in Table 5. The simultaneous presence of the 40 mg absorbent gauze pellet significantly depressed the target granulation tissues, while further significant depression was caused by the pellets impregnated with irritant materials. It was confirmed that the inflammatory reactions surrounding the impregnated pellets were more severe and there was greater exudate than those surrounding the absorbent gauze pellets. In the control animals no "test" implantation was made, but the incision was made and the connective tissue parted in simulation of the trauma of pellet insertion. After five days the connective tissue had not fully repaired but signs of inflammation were barely discernible.

DISCUSSION

In qualitative terms the inflammatory processes which result from the subcutaneous implantation of foreign material are well documented. Even if the material is relatively biologically inert it will become isolated from surrounding tissues, initially by fluid exudate and then by the development of a barrier of granulation tissue. It would be assumed that a more irritant material would, in general, induce a more severe inflammatory reaction. However, the relation between irritant potency and the severity of different components of inflammation do not appear to have been quantitatively defined. This applies also to the interrelation between the inflammatory components themselves, e.g. exudation and the subsequent granulation.

The present investigations have revealed that the presence of irritant material, such as capsicum oleoresin, results in both quantitative and chronological differences

in the development of inflammation, in comparison to the relatively non-irritant cotton fabrics alone. The most marked difference is the potentiation of the degree and persistence of the exudate formation in the presence of the capsicum impregnation. The formation of granulation tissue appeared to be markedly affected by the exudate process, since it first made its appearance at the extremities of the lesions. Thus, in the presence of the copious exudate induced by irritant pellets, the granulation tissue developed first as a rather poorly defined boundary, remote from the irritant focus. As the exudate volume declined, the granulation tissue front advanced in towards the pellet, contact with the pellet being considerably delayed. Even after such contact penetration of the pellet by granulation tissue elements was retarded, in contrast to an almost total colonization of the non-irritant pellets after a similar time. A possible explanation is that residual capsicum oleoresin remained within the pellet, and its influence was revealed both by the much thicker granulation barrier at the surface and the inability of fibroblasts and other cells to survive within the interstices of the cotton fibres.

With these effects of irritant material clearly defined, it is possible to utilize granulation tissue weight data to evaluate irritant potency. This has been applied to investigations of euflavine, boric acid and iodoform, which have been used clinically in medicated gauze fabrics. Such usage is largely obsolete; one reason being the mild irritant properties which retarded wound healing—a process analogous to granulation. Thus, significant increases in both exudate and granulation tissue formation were recorded in the presence of these mild irritants. Even greater and more significant increases were induced by the presence of the capsicum oleoresin, a material with powerful local irritant properties. Such an application, therefore, provides a discriminating test for relative potency of irritants. The value of making both wet and dry weight determinations in assessing granulation tissue growth, has previously been recognized by Penn & Ashford (1963). In conjunction with knowledge of the time course of the inflammatory reactions, such data may give information about both exudation and granulation. In the present case, there was consistent agreement between assessments of relative irritancy obtained by both wet and dry weight data. When pellet granuloma investigations are applied to evaluation of anti-inflammatory properties, inconsistencies between wet and dry weight data might occur, which could be of significance in interpreting modes of action.

The time course of the reactions to normal cotton pellets in the present investigation does not compare closely with either of those described in investigations by Penn & Ashford (1963) or Di Pasquale & Meli (1965). In the former, maximal development of granulation tissue had occurred after two days, and in the latter development continued up to 90 or more days. This disparity serves to emphasize the dangers in basing implantation tests for toxic hazards on absolute evaluations or uncontrolled observations. The differences between the three investigations could be due to variation between animals or laboratory conditions, or to the physical or chemical nature of the implanted materials.

It has been demonstrated that the induction of an inflammatory response in one area of an animal results in an indirect anti-inflammatory influence on other sites of inflammation (Laden, Blackwell & Fosdick, 1958; Di Pasquale, Girerd & others, 1963; Cygielman & Robson, 1963; Goldstein, Shemano & others, 1967). With particular reference to the present investigations, Robinson & Robson (1964) showed that implanted cotton wool or polyester sponge, inhibited the development of cotton

wool granulomata. Their observation that the degree of inhibition varied with the quantity of material implanted, is confirmed by the present results. These findings are consistent with the present observation that the degree of inhibition varies also with the irritant potency of the implanted material. It has been demonstrated that this phenomenon can be used in a test to discriminate between the inflammatory properties of irritant substances and those of relatively inert cotton material. This, however, appears to be less sensitive than tests based upon direct evaluation of granulation tissue formation.

The tests suggested by this investigation might have an increasing variety of applications as new fabrics and materials find their way into surgical and medical use. The main difficulties in such applications would probably be the choice of suitable materials of proven safety or known irritancy with which to make valid comparisons.

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